

## **Arteriovenous patterns in beaked whales**

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### **LONG-TERM GOALS**

To provide a clear picture of the vascular anatomy in beaked whale heads that will facilitate improvement of future nitrogen kinetics models and act as a baseline from which future morphological, pathophysiological and mathematical modeling research can evolve.

### **OBJECTIVES**

The objective of the research thus far was to characterize the gross morphology of the blood vessels in the heads of beaked whales. Description and understanding of this architecture would be accomplished through dissection and diagnostic imaging techniques. Final products would involve peer reviewed publications of resulting data and presentation of findings at the Society for Marine Mammalogy 20<sup>th</sup> Biennial Marine Mammal Conference.

### **APPROACH**

The approach of this project utilized a combination of classical anatomical methods in conjunction with new diagnostic imaging modalities in order to decipher the complex vascular patterns in the heads of beaked whales. The aforementioned approach used vascular perfusion mixture of liquid latex and a radiopaque contrast agent. After injection into the blood vessels and adequate curing time, specimens were imaged via computed tomography (CT) using a thin slice protocol. Resulting DICOM data was postprocessed using Amira<sup>TM</sup> (VSG Imaging) software and data were examined prior to commencement of gross dissection. After sufficient review of CT data, each specimen underwent a detailed gross dissection of the injected vascular system. Important findings were photo and video documented. When warranted, injected and/or uninjected structures were excised from the specimen, preserved in formalin and re-examined more closely. All work to date has been carried out by the Principal Investigator (PI). In addition to the PI (Alexander Costidis), an assistant researcher (Sentiell Rommel, Ph.D.) was also hired from the University of North Carolina Wilmington to conduct the work. Dr. Rommel has assisted with all experimental methodologies and interpretation of the resulting observations and is currently collaborating with the PI to generate the first two peer-reviewed publications.

### **WORK COMPLETED**

To date, the first of two distinctly separate research objectives has been met. The PI and assistant researcher completed their injections, imaging and dissections of beaked whale specimens in New Zealand, and have substantially increased their understanding of the vascular patterns in the heads of

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numerous beaked whale species. The experimental phase of this objective has now been completed. Eleven beaked whale specimens (7 *Mesoplodon grayi*, 2 *Ziphius cavirostris*, 1 *M. layardii*, 1 *M. bowdoini*) were obtained while in NZ. With the exception of a neonatal *Z. cavirostris*, all specimens consisted of heads transected either at the atlantoccipital (neck) joint or at the level of the first three cervical vertebrae. Owing to the fact that none of the specimens were collected with our experimental protocol in mind (all except one had been previously collected), most specimens had been severed at an oblique ventral angle which resulted in removal of the parent cervical arteries (common carotid) and veins (common jugular). Of the eleven specimens, three (1 adult male, 1 adult female, 1 neonate female) were of higher tissue quality (e.g. less decomposed) and yielded more detailed results. The remaining specimens were of variable quality due to the method of collection and time in cold storage, and therefore yielded varying results. Nonetheless, all specimens examined were beneficial to the process and added various layers of detail to the subset of high quality specimens. Due to the limited availability of high quality, intact specimens, the two best adult specimens (*M. grayi*) were used as the primary specimens for imaging of blood vessels. The adult male was used for arterial imaging and the adult female for venous imaging. The neonate (*Z. cavirostris*) was used for combined arteriovenous imaging. The remaining specimens were used either for simple gross dissection or for gross dissection following vascular injection of latex without contrast. All specimens were imaged under computed tomography (CT) prior to any experimental protocol and one of the higher quality specimens also underwent magnetic resonance imaging (MRI). This proved exceedingly fruitful since the adult female that was imaged for the venous system provided important insights into the spatial associations and dynamic nature of the pterygoid air sinus and adjacent venous lake (see description below). The PI and assistant researcher are currently conducting post-processing of data obtained through contrast angiographic CT and gross dissection and are in the process of generating the first publication to be submitted in Fall 2013. An abstract on the findings from the first research objective has been accepted and will be introduced as an oral presentation at the upcoming 2013 SMM (Society for Marine Mammalogy) Biennial Marine Mammal Conference in Dunedin, NZ.

## RESULTS

The extracranial arterial and venous morphology was successfully examined in a number of beaked whale species. The primary species examined due to specimen availability was the Gray's beaked whale (*Mesoplodon grayii*), however numerous other species including Cuvier's (*Ziphius cavirostris*), Straptoothed (*M. layardii*) and Gervais' (*M. europaeus*) were also examined and provided a comparative framework. The most important result from the research conducted in New Zealand was a significantly improved understanding of anatomy of the arteries and veins in the head (Figs. 1&2). This most basic knowledge which until recently was lacking, feeds into a better understanding of the architectural and physiological composition of these extreme divers. The vascular information can inform models of diving gas kinetics (e.g. nitrogen saturation and supersaturation) as they may pertain to decompression sickness (DCS) as well as help with the understanding and localization of pathophysiological processes such as gas and fat embolization. In addition to generating a more generalized picture of the vascular morphology in the head of beaked whales, specific structures of great interest were discovered. We believe that these structures suggest significant functional implications and may provide important insights into the sources of some of the lesions observed in strandings consistent with DCS.

Specifically, we found four structures which we believe to be of great significance, and whose arteriovenous associations have either not been previously described or have been described in limited scope. The first structure is found in the pterygoid region (Fig 2) on the bottom of the skull. This region has been described in detail in numerous delphinid species and has been shown to have intimate

associations between its accessory air sinus system and the pterygoid vasculature (Costidis & Rommel, 2012; Fraser & Purves, 1960). Although a similarly extensive juxtaposition of pneumatic and vascular spaces exists in beaked whales, the differences in the structures relative to other toothed whales are striking. As has been previously reported, bottlenose dolphins possess an accessory sinus system with numerous outpocketings that extend in various directions from the submaxillary region all the way back to and around the tympanoperiotic earbone complex (Fraser & Purves, 1960; Houser et al. 2001). These various air sacs are lined with intricate venous plexuses or networks of small veins that interconnect in a seemingly random manner. The plexuses are extensively connected to the rest of the venous system of the head and therefore have numerous potential drainage and filling routes. Although this morphology is best studied in the bottlenose dolphin, many other toothed whales appear to have similarly composed and oriented structures, including the deep-diving sperm whales and pygmy sperm whales. Surprisingly, it appears as though beaked whales have taken a relatively different approach to these structures. Although they too have large air sacs in the pterygoid region and those air spaces are associated with extensive vascularization (Fig. 3), the geometry and composition appear quite different. As was already known, the accessory sinus system is much less elaborate, lacking the numerous outpocketings seen in other toothed whales. Instead the pterygoid sinus forms a single large pocket of air that connects directly to the air spaces in and around the earbone complex. Similarly, instead of being composed of an intricate meshwork of small veins, the venous plexus surrounding the air sacs is in fact a single large venous lake whose continuity is disturbed by only the numerous delicate, transparent baffles of its trabeculae (Figs. 3&4). We found it significant that many of the delicate baffles contain small arteries originating from the pterygoid arteries (Fig. 5). This morphology results in small arteries bathed in a venous lake (Fig. 6), and may be capable of exchange of diving gases. We feel this is an important finding because nitrogen absorption in the lungs has long been thought to cease at depth when alveolar collapse occurs. The juxtaposition of the arteries and venous lake could form an alternate site of absorption that increases at depth due to diminishing air volume in the adjacent pterygoid sinus and increasing volume in the pterygoid venous lake. CT scans performed before and after injection of latex into the venous system showed a compensatory compression of the pterygoid sinus in response to engorgement of the pterygoid venous lake (Fig. 7), suggesting that the two structures do indeed have a complementary filling and emptying function as was originally proposed by Fraser & Purves (1960).

The second anatomical finding we believe to be significant was with respect to the vascular anatomy in the lower jaw. As was expected based on studies of the bottlenose dolphin and other species (Costidis & Rommel, 2012), the intramandibular jaw fat (IMFB)—the acoustic fat body within the hollow lower jaw bone—was invested with an extensive venous plexus (Fig. 8). The acoustic fat forming the supportive matrix of the venous plexus does so in a trabecular fashion, in effect creating an intricate structure of fatty tubes and trellaces around which the venous blood of the plexus travels. Interestingly, many if not most of those trabeculae contain small arteries (Fig. 9), creating a structure in which there appears to be considerable arterial investment of the acoustic lipids followed by subsequent immersion of those lipids in venous blood (Fig. 10). Koopman & Westgate (2012) showed that acoustic fats tend to have high nitrogen saturation coefficients and therefore are capable of absorbing considerable nitrogen from the arterial system. Such a morphology in the acoustic fat bodies might seem contrary to reason, as one might think that isolating such an important sensory structure from nitrogen absorption would be a safer strategy, however the voluminous juxtaposition to the venous system may under normal circumstances help mitigate deleterious effects by facilitating rapid unloading of nitrogen from those tissues. We also found interesting the fact that the walls of the venous plexus within the IMFB appeared exceedingly thin considering the large caliber of the veins. Preliminary observations suggest that the venous walls are composed of only a thin basement

membrane and endothelial layer yet the veins themselves are the size of venules and veins. Such thin walls may suggest a specific modification that facilitates re-absorption of nitrogen and offloading out of the IMFB during ascent from a dive.

Our most intriguing finding was that unlike dolphins in which the pterygoid plexus has countless connections to the rest of the venous system, the large pterygoid venous lakes of beaked whales appear to have only two connections to the rest of the cephalic venous system. This would suggest that collateralization of venous flow is minimal and only two meaningful inflow and outflow tracts exist through which filling and emptying can occur. Given the large size of the venous lakes and their adjacent air sinuses, this raises important questions regarding the rate of filling and emptying and whether that may play a role in the pathogenic processes observed in DCS-like cases of strandings (Fernandez et al, 2005). The two aforementioned connections occur at the ventrocaudal and dorsolateral aspects of the venous lakes. The ventrocaudal connection is to the pterygoid vein and directly to the jugular system, however the pterygoid vein travels through a seemingly undistensible fibrovenous plexus. Therefore a portion of the pterygoid vein appears incapable of expanding to receive an increased flow rate. The dorsolateral connection (Fig. 8) is plexiform and to the IMFB plexus which as previously discussed is composed of delicate strands of acoustic fatty tissue. We hypothesize that during an inappropriately rapid ascent from depth, the air volume within the pterygoid sinus must expand rapidly and consequently pressurize the blood within the adjacent venous lake (Fig. 7). The venous blood must therefore rush out of the two aforementioned connections and since the ventrocaudal connection is likely incapable of expanding, much of the blood is likely to get forced into the delicate IMFB plexus. Such hemodynamic alterations may help explain a number of the pathologic observations in beaked whales exhibiting DCS-like lesions. Firstly, such forceful ejection of blood out of the pterygoid venous lakes and into the IMFB could effectively disrupt the thin venous walls and lead to the observed IMFB hemorrhages. Disruption of the venous wall and damage to the acoustic fats could subsequently allow for introduction of fat emboli into circulation, thereby resulting in the systemic distribution of intravascular fat emboli previously observed in beaked whales (Fernandez et al, 2005). Similarly, since the outflow channels for the large pterygoid venous lakes are limited in their volume this anatomy could help explain some of the hemorrhages observed in the ears and brains of beaked whales. If the pterygoid venous lakes are incapable of rapidly emptying in response to increasing air volume in adjacent pterygoid and peribullar sinuses, a rapid ascent could result in increasing pressurization of the air sinuses. The increasing pressures could result in physical disruption of the auditory system as well as transference of the pressures through the skull foramina and into the brain case.

The last finding of considerable interest relates to thermoregulation. We observed a sizable arteriovenous structure suggestive of counter-current heat exchange (CCHE) not only in the lingual and oral regions (Figs. 11-12) but also along the maxillary (Figs. 13-14) and submaxillary regions of the rostrum. In fact, all peripheral arteries of the head were to some degree either tightly juxtaposed to or completely encased in venous structures. The lingual CCHE has previously been described in gray whales (Heyning 2001) but its presence has not been discussed in toothed whales nor has it been imaged as clearly. We found the orolingual CCHE to be composed of two relatively distinct structures which likely form the basis of the alternate heating and cooling function. An extensive but loose venous plexus was found lining the oral cavity and a dense and thick venous plexus was also found along the palatine bone (Fig. 2) on the roof of the mouth. Both of these structures are suggestive of a thermal cooling function by juxtaposing large venous structures with high surface area to surfaces in contact with cold water. An analogous structure was described on the palatine surface of the bowhead whale (Mead, Werth and George, 2013). A similarly extensive venous plexus was found along the

superficial surfaces of the tongue and gular region. Conversely, in the deep tissues of the tongue were the large lingual and sublingual arteries which were encased in venous plexuses (Fig. 12) nearly identical to the thermoregulatory peri-arterial venous retia previously described in the dorsal fins and flukes of cetaceans (Elsner 1966; Scholander & Schevill, 1955). These structures are strongly suggestive of heat conserving CCHE structures. The functional picture that forms when these structures are considered together is that there are numerous vascular structures in the head which can be employed to either dump heat (superficial venous plexuses) or conserve heat (lingual CCHE).

## IMPACT/APPLICATIONS

The current findings significantly increased our understanding of the most basic morphological and structural composition of the head of beaked whales. Future impacts of our findings may depend on the level of commitment to increasing the detail and scale of our understanding of the aforementioned anatomy. The current results provide important insights into potential rheological and pathophysiological processes that may contribute to DCS-like lesions observed in some stranded beaked whales. These insights may prove useful when interpreting lesions in future postmortem examinations and may also provide a springboard for future functional (e.g. studying blood flow patterns during altered pressure profiles), morphological (e.g. integrating gross and microscopic vascular anatomy into a system-wide picture of vascular associations to important tissues) and mathematical (e.g. modeling of nitrogen absorption and elimination) studies. Understanding the differences in this morphology from a comparative perspective (e.g. shallow vs. deep-diving cetaceans, delphinids vs. physeterids vs. ziphiids, etc.) may also shed light on the functional consequences of the morphology. Future mathematical models of nitrogen saturation should consider the aforementioned vascular morphology, especially when including variables such as blood compartments (e.g. volume, location, etc.) and nitrogen kinetics (e.g. nitrogen absorption at pterygoid sinus and IMFB). Although these findings should inform future models, more detailed quantification of certain physical variables such as microvascular densities in the intramandibular fat bodies and wall thicknesses of blood vessels and sinuses (e.g. barriers to nitrogen diffusion, etc.) should significantly improve the accuracy of future mathematical models.

## RELATED PROJECTS

A grant proposal has been submitted requesting funds to study the histologic, ultrastructural and microvascular details of some of the vascular regions which this current study found to have potentially important functional and pathological implications.

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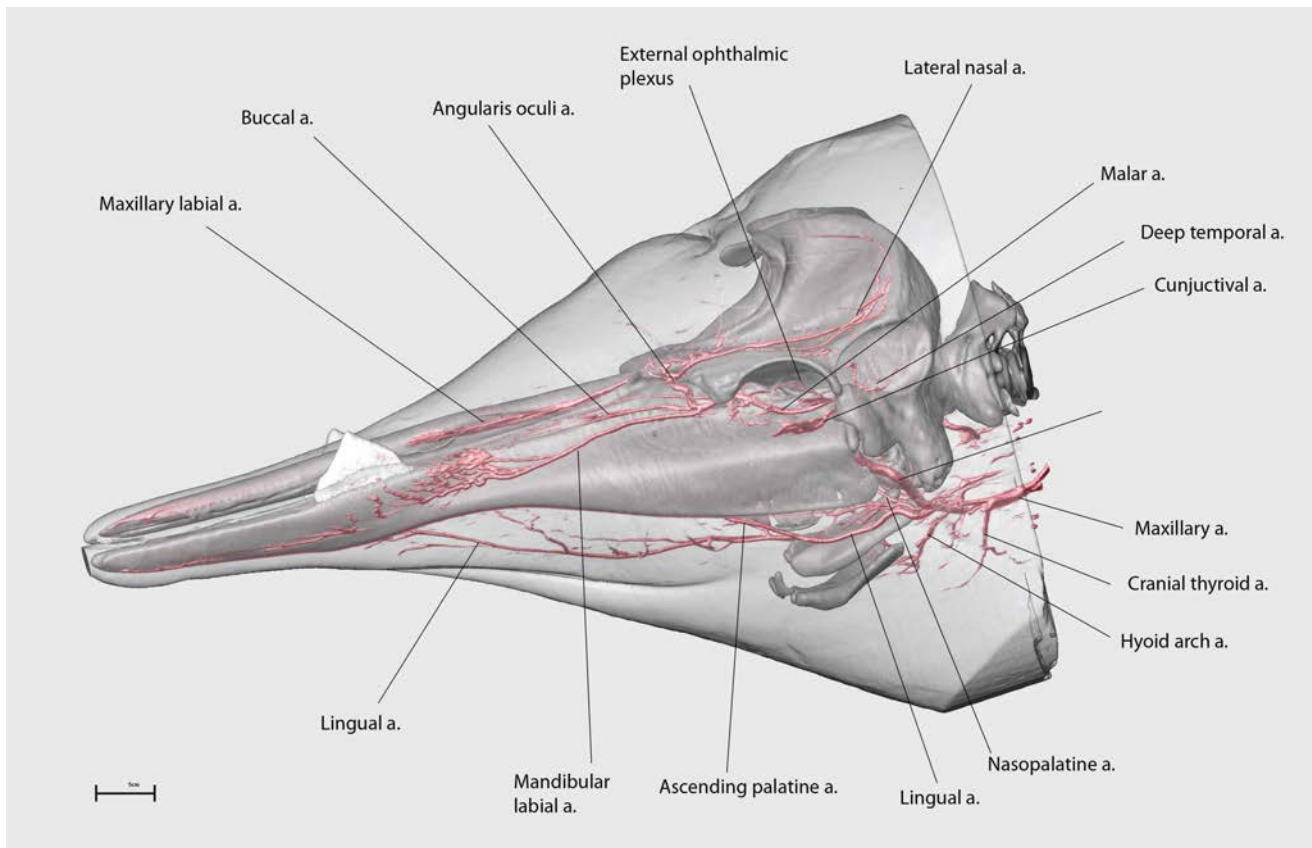


Figure 1: Left lateral view of three-dimensional reconstruction of the arteries (red), skull and blubber in the head of an adult male Gray's beaked whale. The major arteries that were identified are labeled accordingly.

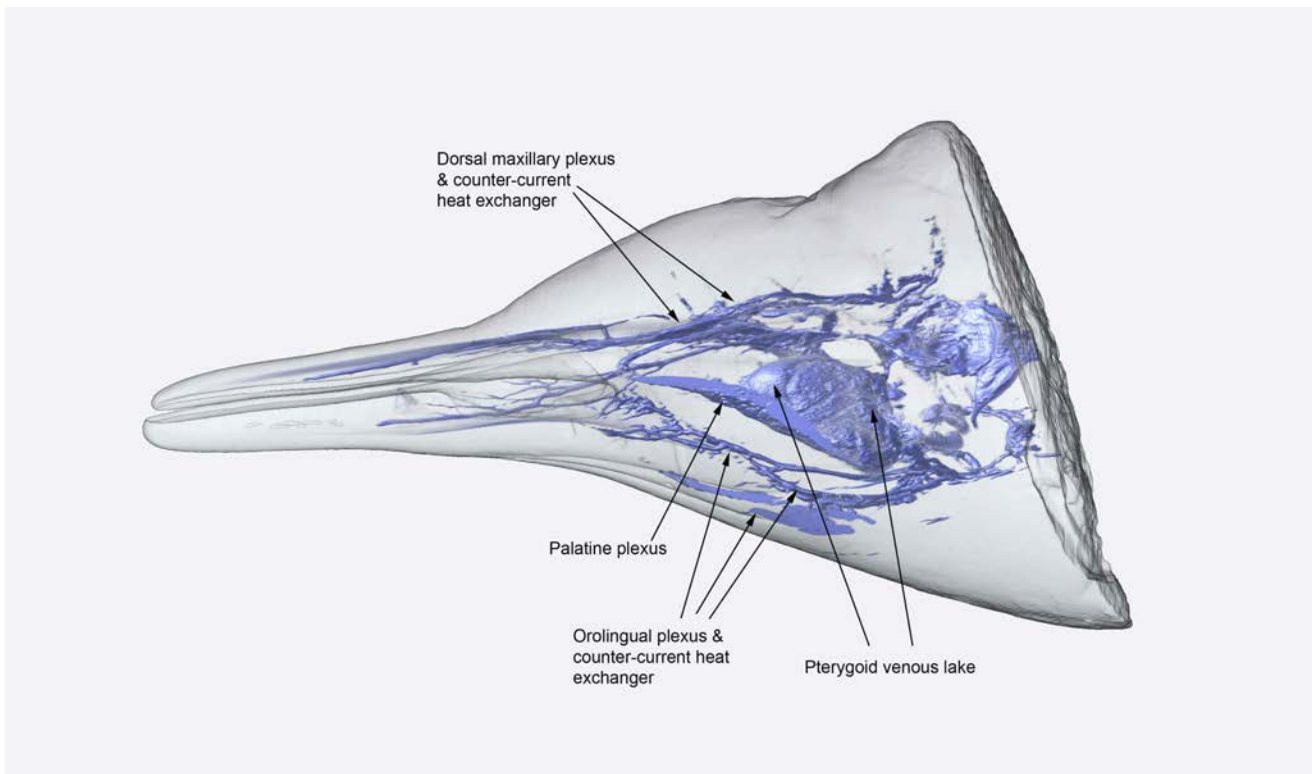


Figure 2: Mid-sagittal view of three-dimensional reconstruction of the blubber and the veins (blue) on the right side of the head of an adult female Gray's beaked whale (*Mesoplodon grayi*) showing the regions of main interest labeled. Note the large volume of the pterygoid venous lake which is juxtaposed to the pterygoid air sinus.



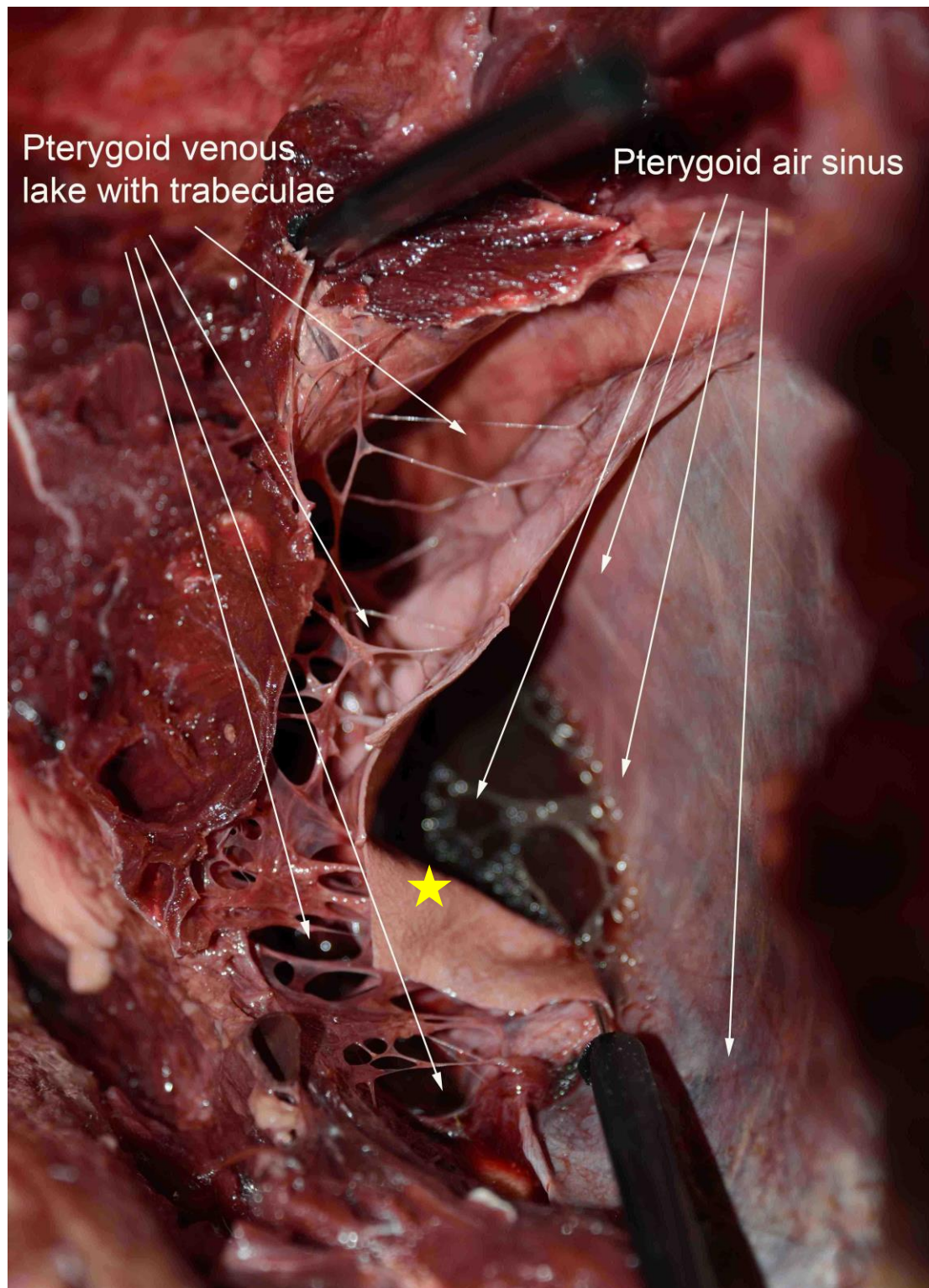


Figure 3: Cross-sectional view of the pterygoid venous lake and air sinus complex of a Gray's beaked whale showing tight juxtaposition of the venous lake to the air sinus. Based on pre- and post-venous filling imaging, the filling and emptying of the two structures appears to be inversely complementary. Compression of the air sinus leads to filling of the venous lake, while expansion of the sinus leads to emptying of the lake. Note the relatively thin lining (yellow star) separating the two structures.



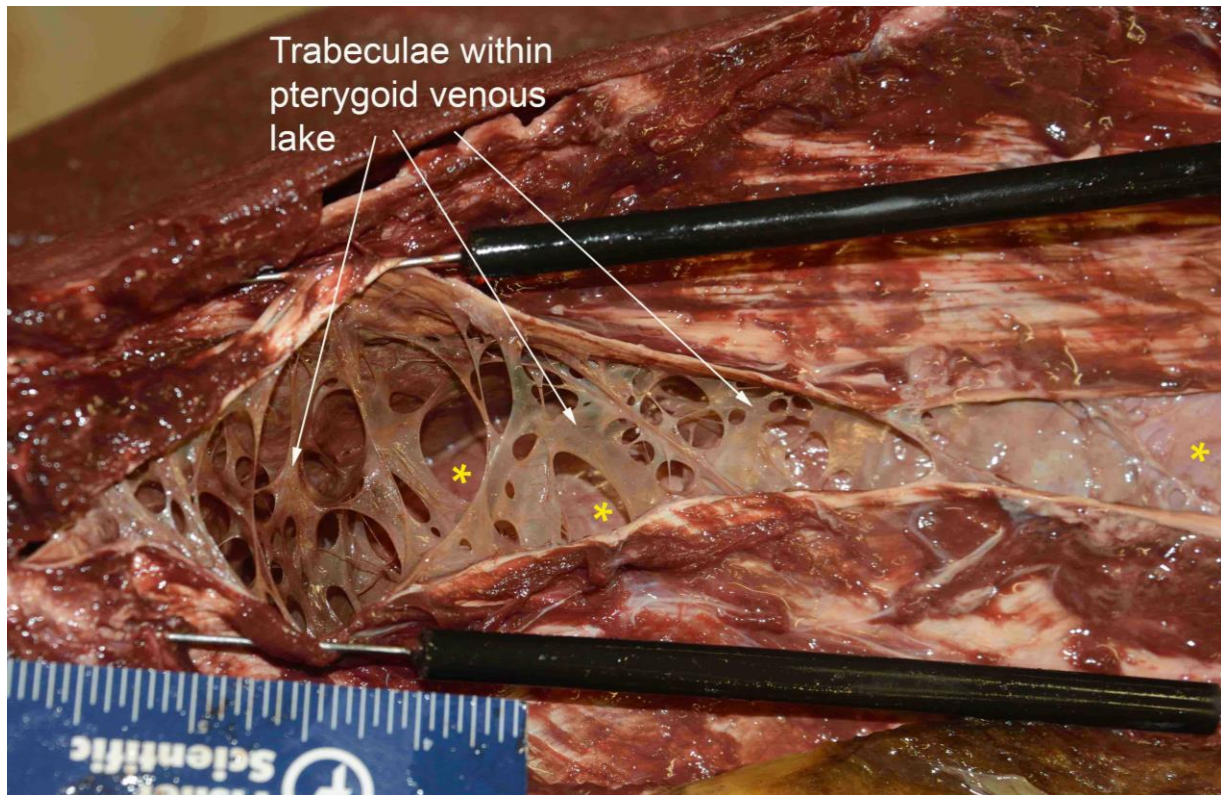


Figure 4 : Ventral view of the pterygoid region of a straptoothed whale with the tendon of insertion of the pterygoid muscle transected to expose the delicate trabeculae of the pterygoid venous lake. Note the lining of the pterygoid air sinus (asterisks) immediately juxtaposed behind the venous lake. (ONR Award #: N000141210656)

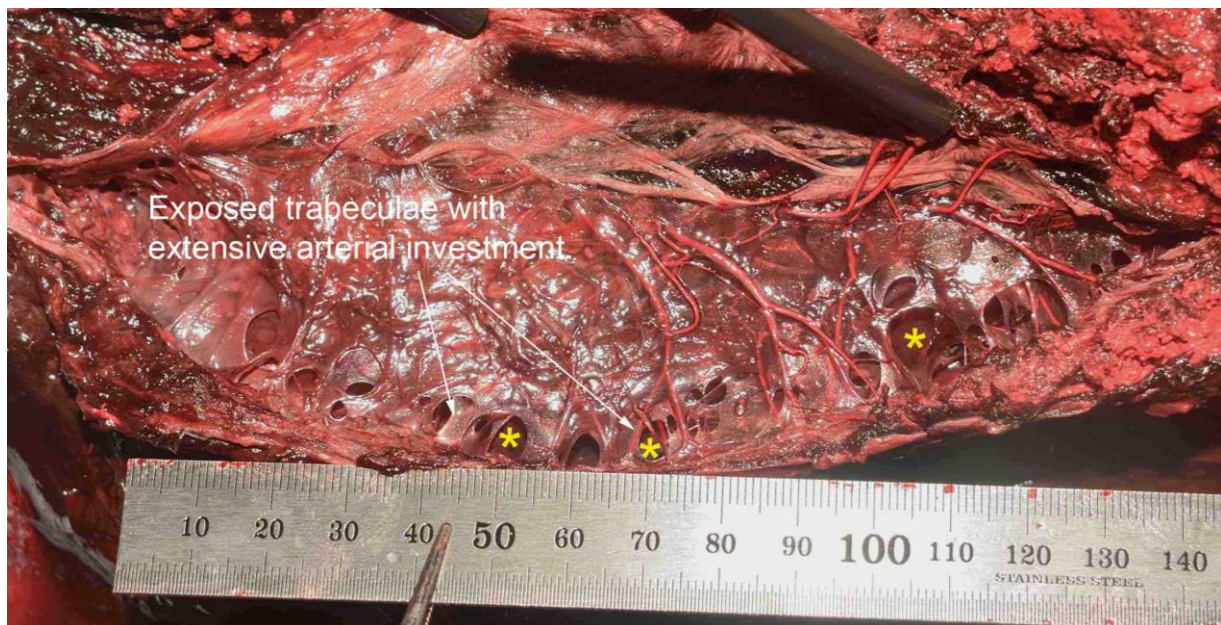


Figure 5: Lateral view of the delicate trabeculae within the pterygoid venous lake of a Gray's beaked whale. Note the countless arteries coursing through the thin-walled trabeculae and the spaces surrounding the trabeculae. The spaces (asterisks) represent the venous spaces of the pterygoid venous lake, and under certain circumstances are filled with venous blood that bathes the trabeculae. Many of the finer arteries remain uninjected, suggesting that an even greater density exists. (ONR Award #: N000141210656)



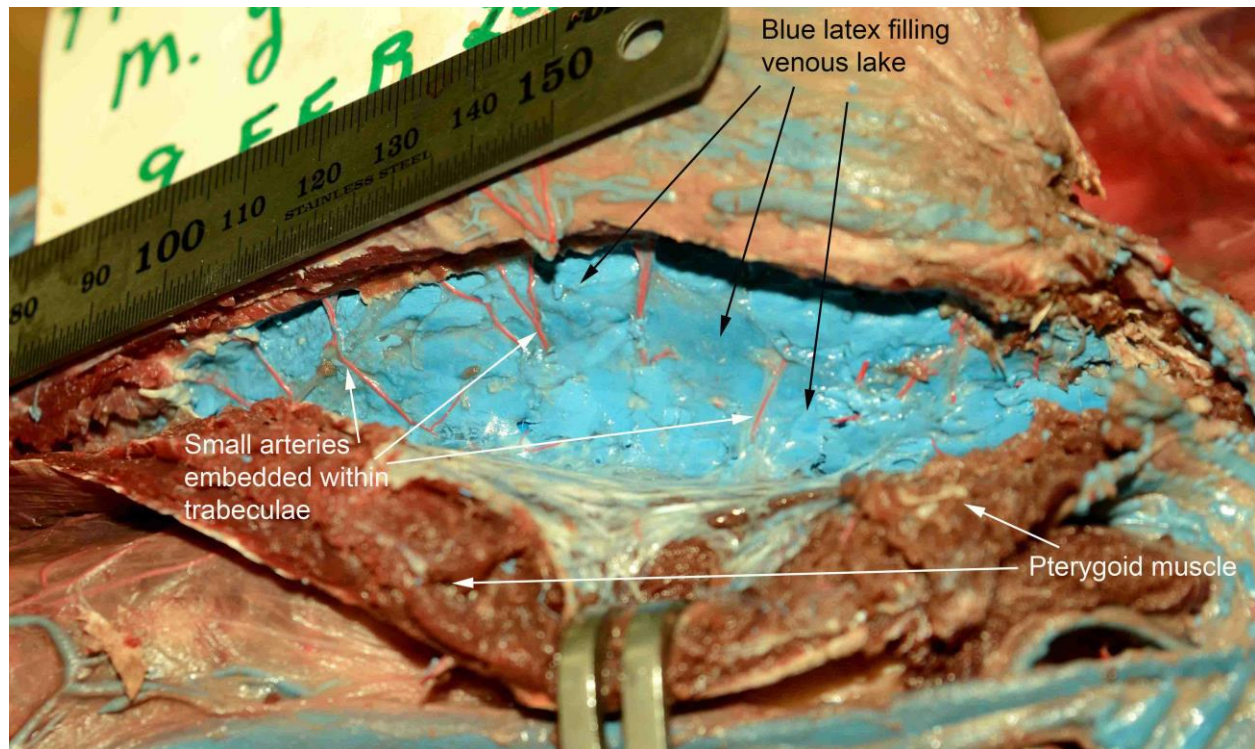
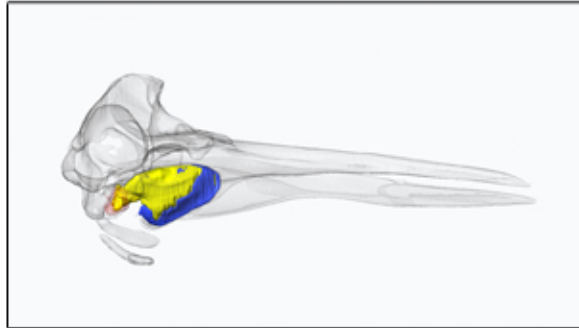
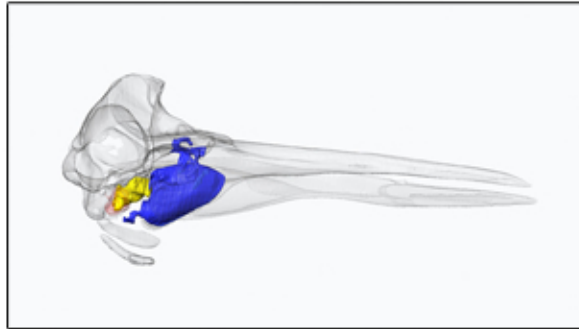


Figure 6: Ventrolateral view of the pterygoid region of a Gray's beaked whale with the pterygoid muscle transected and retracted to expose the venous lake filled with blue latex and the pterygoid trabecular arteries filled with red latex. Note how the small arteries and their delicate trabeculae are completely bathed in blue latex (venous blood). (ONR Award #: N000141210656)

Surface

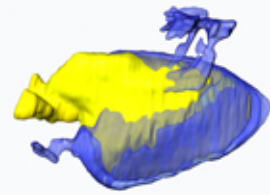


Lateral view

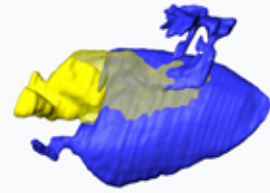


Dive

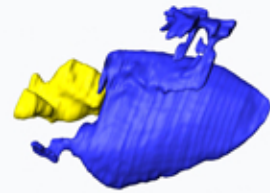
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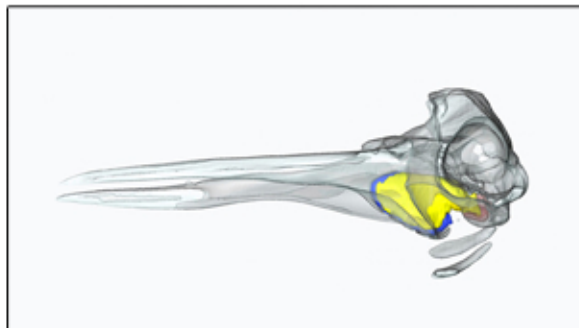
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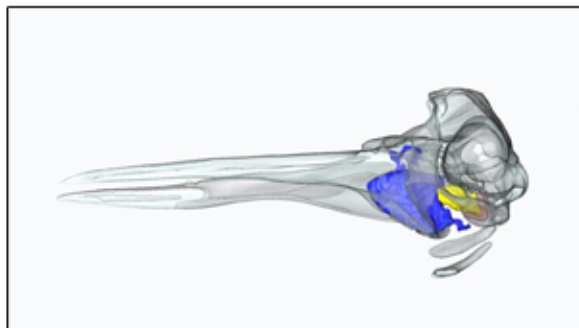
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Surface



Medial view

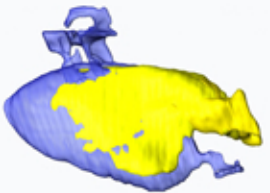


Dive

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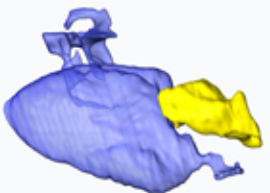


Figure 7: Medial view of the pterygoid venous lake and adjacent accessory air sinus system. Note the compensatory reduction in air volume in the sinus due to filling of the venous lake. This condition likely emulates the relationship during descent and ascent from a dive. During descent when the air sinus is compressed and shrinks, the venous lake likely fills in response. Conversely, upon ascent as the air in the sinus expands the venous lake is likely forced to drain.

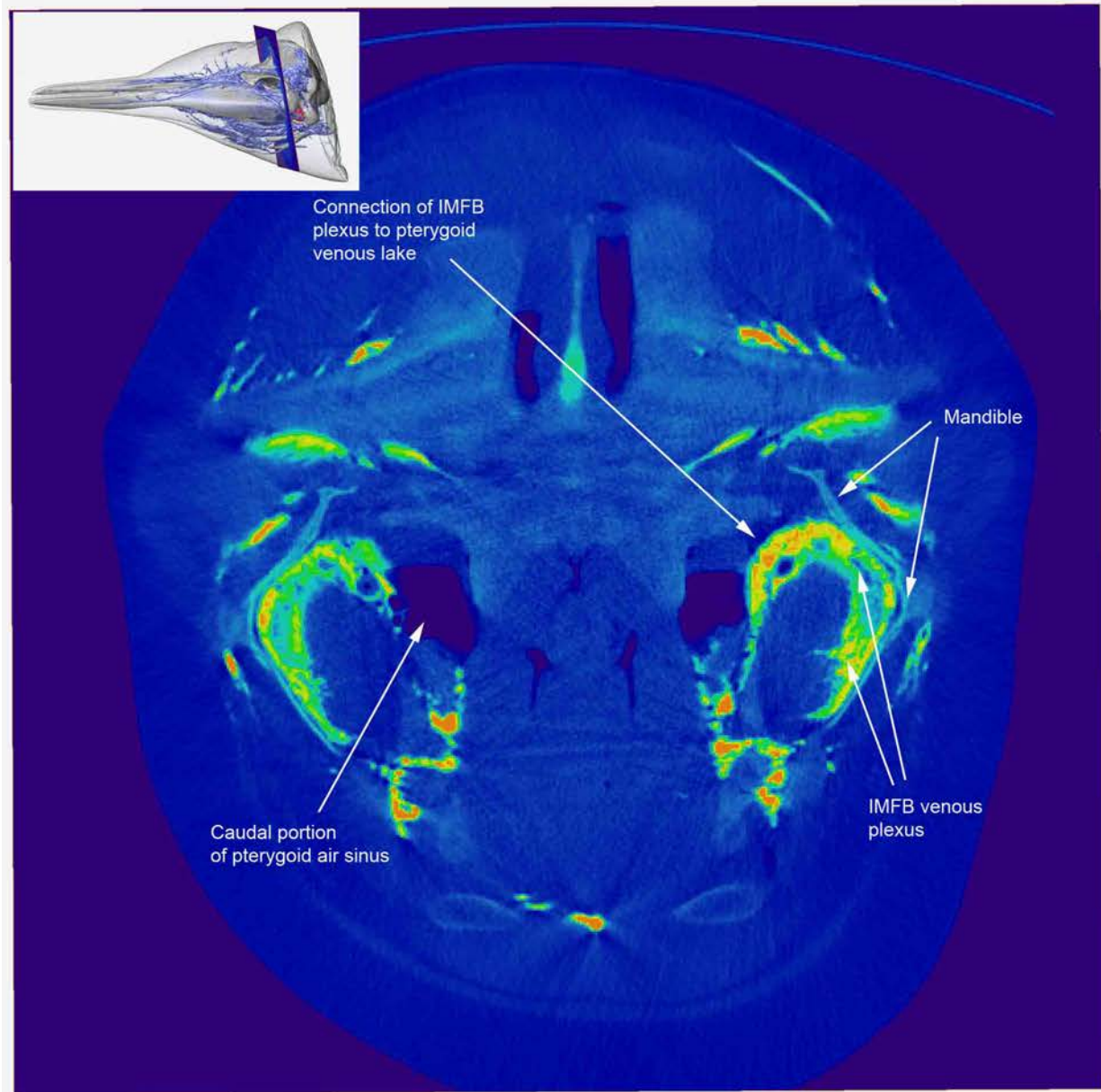


Figure 8: Cross-sectional view of contrast enhanced venous system from computed tomographic angiogram of an adult female Gray's beaked whale head. Note the heavy investment of the intramandibular fat body (IMFB) with a venous plexus injected with contrast medium (bright region medial to mandible). Insert at top left shows the location of the plane of section.



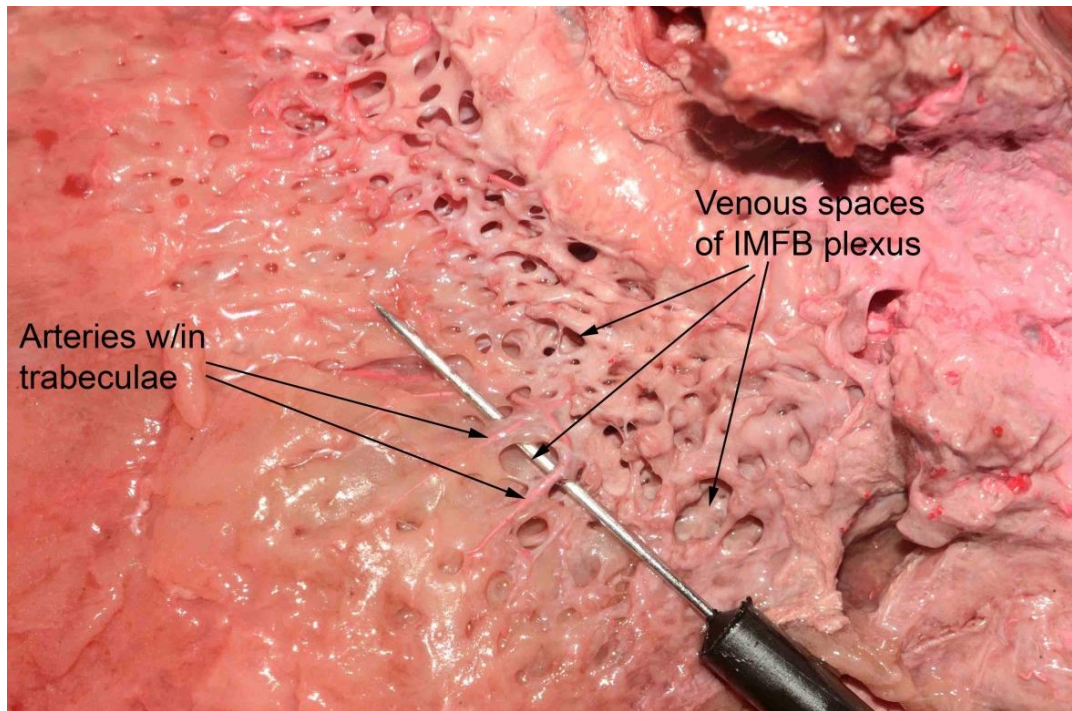


Figure 9: Lateral view of gross dissection of intramandibular fat body of a Gray's beaked whale (*Mesoplodon grayi*) injected with red latex in the arterial system. The probe is placed within the spaces comprising the venous plexus. Those venous spaces surround the countless trabeculae (supporting strands) some of which are being elevated by the probe. Note the transition of the dense connective tissue trabeculae to translucent, fatty trabeculae composed of the acoustic fat and small arteries coursing through them. The trabeculae are completely surrounded by venous spaces and therefore bathed by the venous blood. (ONR Award #: N000141210656)

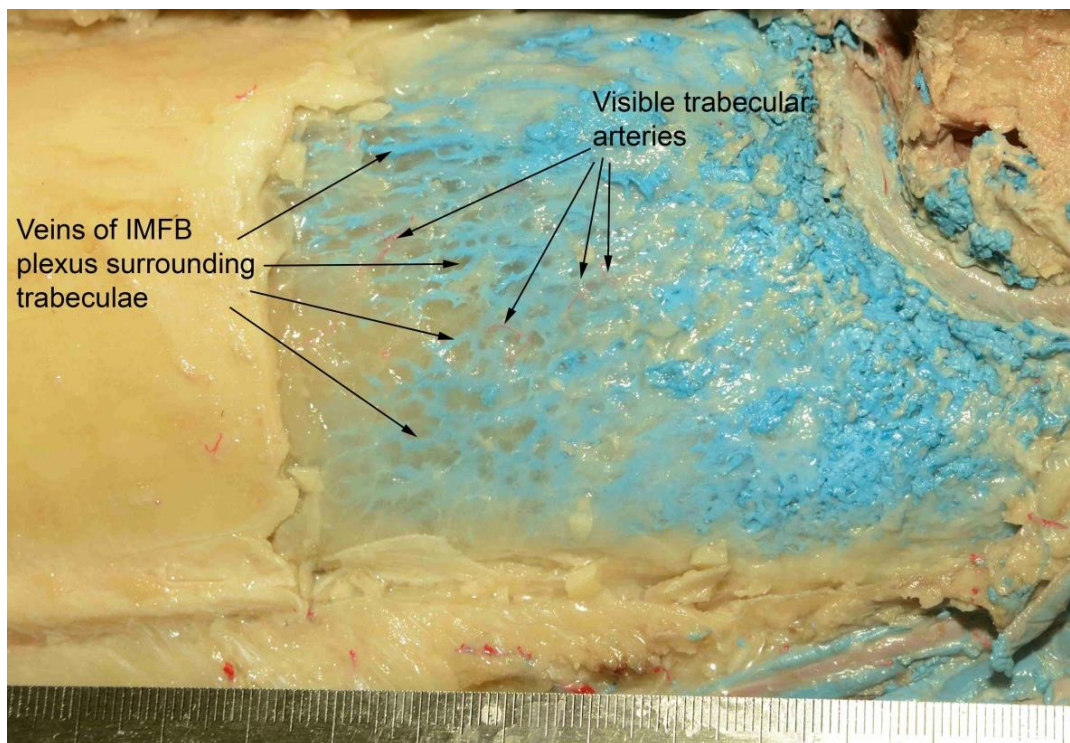


Figure 10: Lateral view of gross dissection of intramandibular fat body of a Gray's beaked whale injected with red latex in the arterial system and blue latex in the venous system. Note that the spaces observed in Figure 3 are filled in with blue latex, and that the few small trabecular arteries visible are embedded in the venous plexus. (ONR Award #: N000141210656)



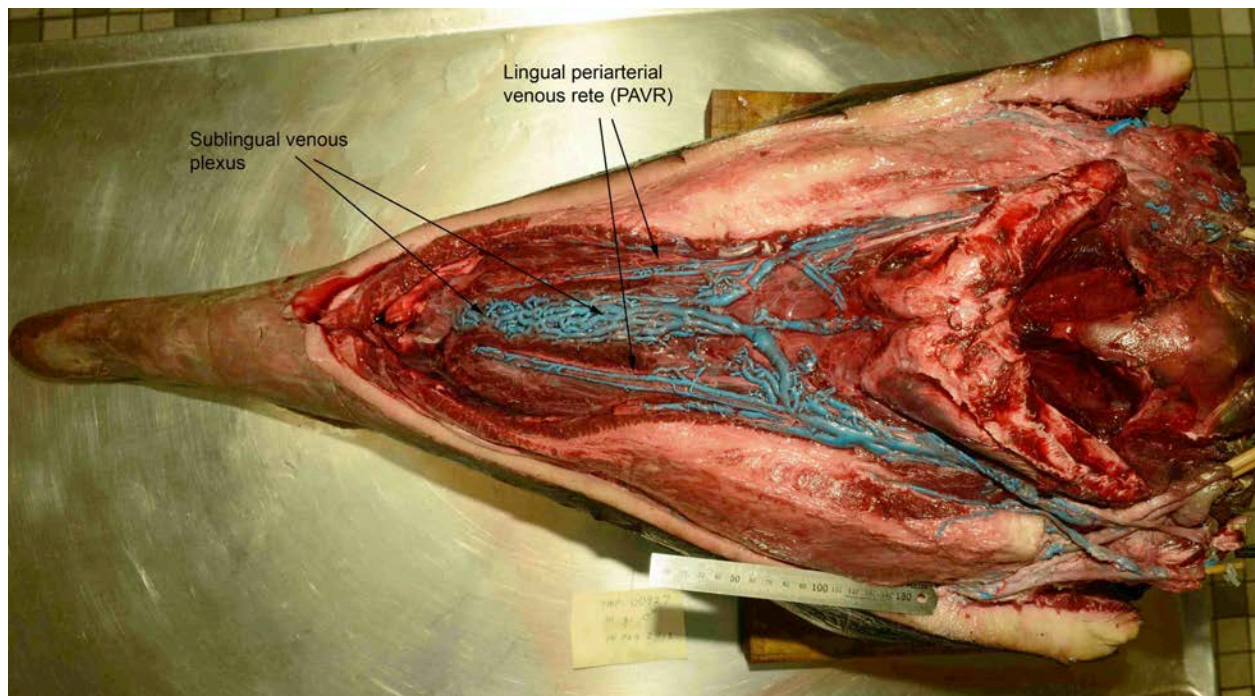


Figure 11: Ventral view of an adult male Gray's beaked whale showing injected veins (blue) and arteries (red) of the superficial sublingual venous plexus (cooled venous return) and the periarterial venous retia (warmed venous return).

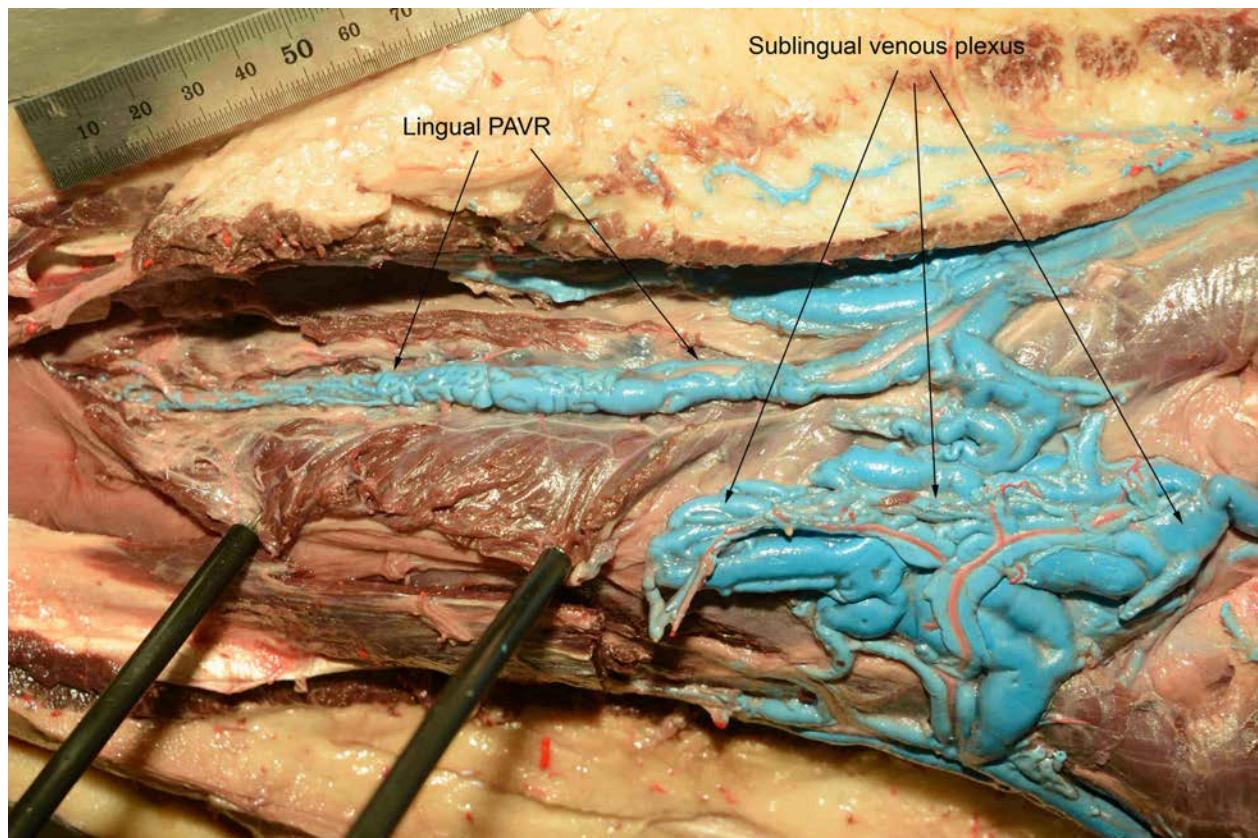


Figure 12: Closeup ventral view of orolingual counter-current heat exchanger showing superficial (ventral) sublingual venous component and deep (dorsal) peri-arterial venous retia (PAVR). This arrangement is suggestive of a superficial cooled venous return and a deep warmed venous return. Note the similarity of the PAVR structures to those described by Sholander & Schevill (1955) and Elsner (1966) in the extremities of cetaceans.



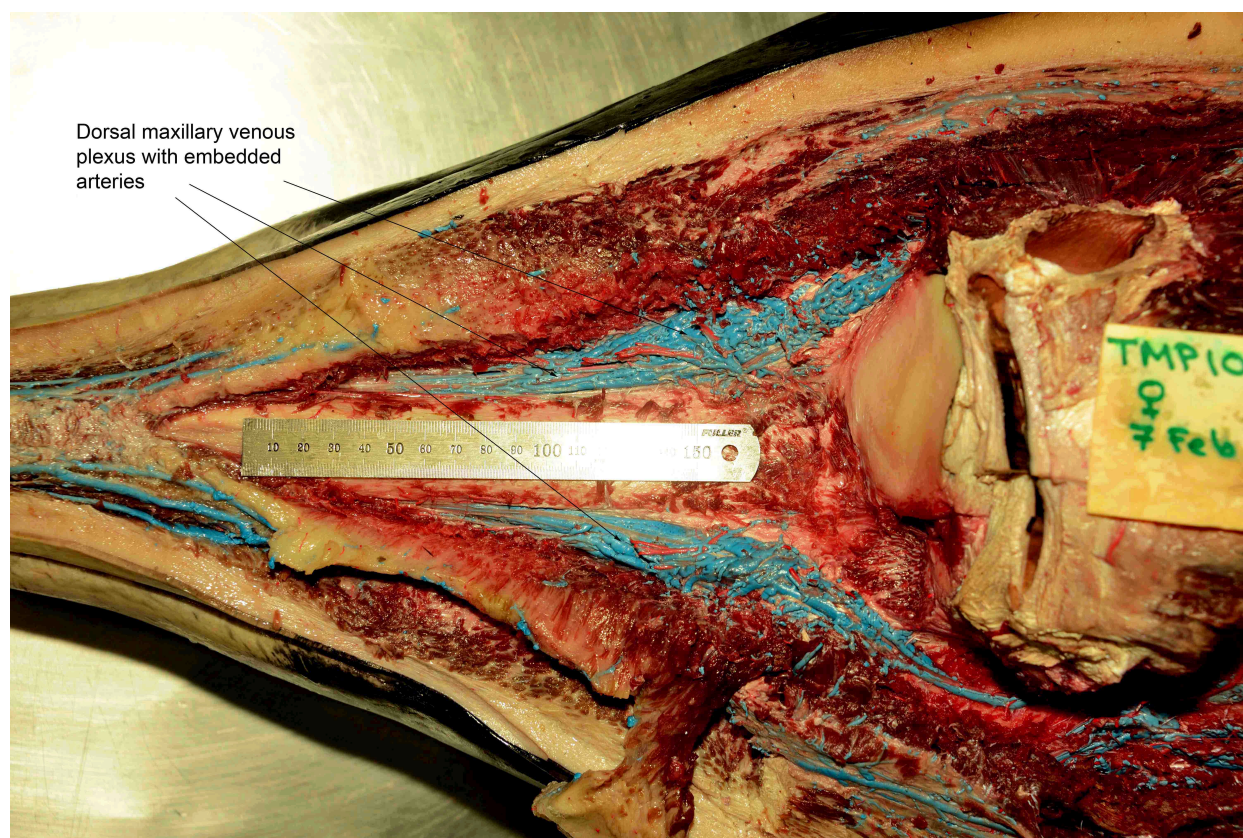


Figure 13: Dorsal view of dorsal maxillary plexus showing intricate arrangement of veins encasing arteries in a typical counter-current heat exchange pattern.

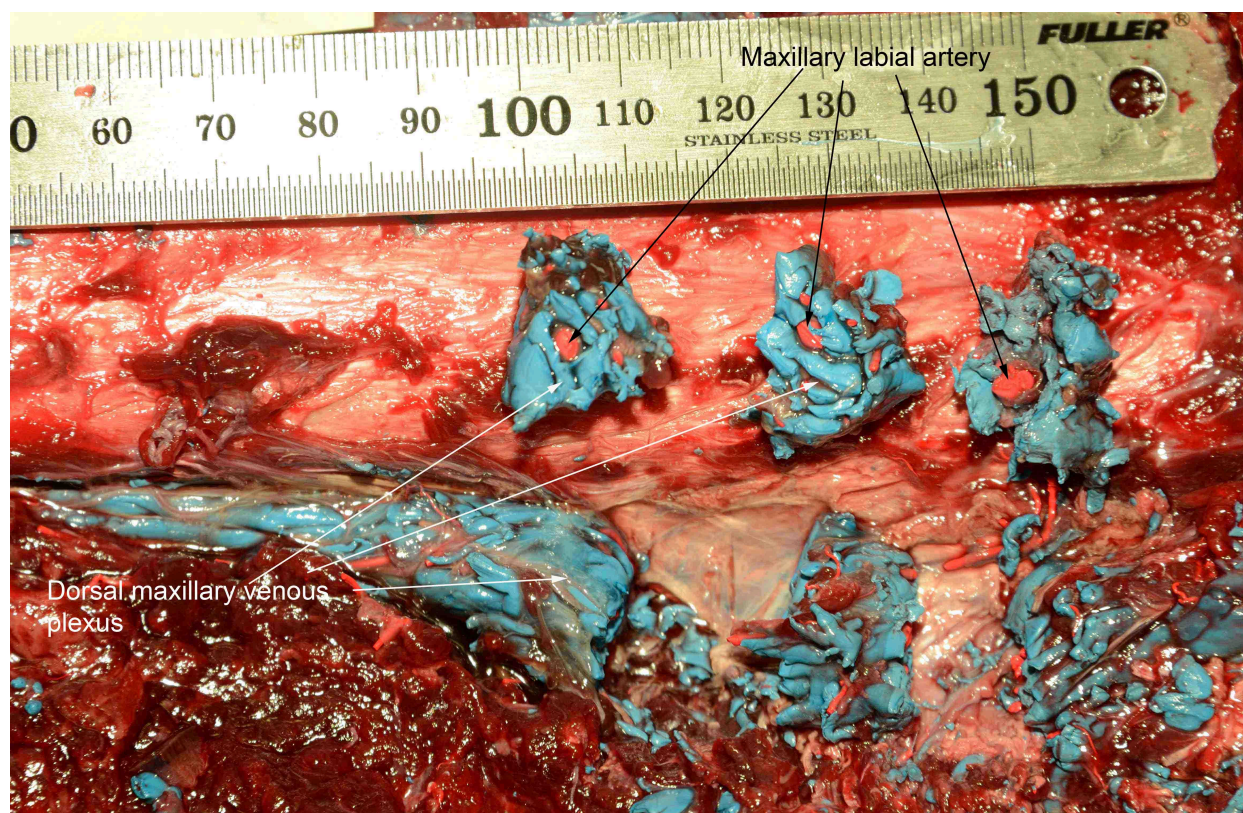


Figure 14: Cross sectional view of the dorsal maxillary venous plexus showing the maxillary labial artery and its tributaries encased within the venous plexus.